



09/940,235, 04-09-02 4

Attorney's Docket No.: 07064-009002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Rajesh Kumar, et al. Art Unit : 1652
Serial No. : 09/940,235 Examiner : Unknown
Filed : August 27, 2001
Title : NOVEL CLOT-SPECIFIC STREPTOKINASE PROTEINS POSSESSING
ALTERED PLASMINOGEN ACTIVATION CHARACTERISTICS AND A
PROCESS FOR THE PREPARATION OF SAID PROTEIN

BOX SEQUENCE

U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

PRELIMINARY AMENDMENT

In response to the communication dated October 5, 2001 (copy enclosed), applicants submit herewith a Sequence Listing in computer readable form as required by 37 CFR §1.824. In addition, applicants submit an initial Sequence Listing as required under 37 CFR §1.823(a) and a statement under 37 CFR §1.821(f).

Applicants respectfully request entry of the paper copy and computer readable copy of the Sequence Listing filed herewith for the instant application. Furthermore, applicants request entry of the following amendments.

In the specification:

Insert the paper copy of the Sequence Listing filed herewith following the Oath/Declaration.

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the U.S. Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202

4/5/2002
Date of Deposit

Signature

NERISSA M. PARIS

Typed or Printed Name of Person Signing Certificate

Replace the paragraph beginning at page 12, line 7, with the following rewritten paragraph:

--**Fig. 3.** DNA and protein sequence of streptokinase of *S. equisimilis* H46A (SEQ ID NOs:1 and 2, respectively) (GenBank accession number: gb/K02986/STRSKC).--

Replace the paragraph beginning at page 12, line 16, with the following rewritten paragraph:

--**Fig. 6.** DNA and protein sequence of the gene-segment encoding for FBDs 1-5 of human fibronectin (SEQ ID NOs:3 and 4, respectively) (the DNA sequence has been obtained from EMBL; the file and accession no.'s are ID-HSFIBI and X02761, K00799, K02273, X00307, X00739).--

Replace the paragraph beginning at page 12, line 32, with the following rewritten paragraph:

--**Fig. 11.** Nucleotide sequence of SK-NTRN gene (SEQ ID NO:5).--

Replace the paragraph beginning at page 12, line 34, with the following rewritten paragraph:

--**Fig. 12.** Predicted secondary structure of native (A) and translationally silently modified (B) 5'-ends of the SK gene sequence (SEQ ID NOs:27 and 28, respectively).--

Replace the paragraph beginning at page 13, line 7, with the following rewritten paragraph:

--**Fig. 14.** Nucleotide sequence of SK-NTR gene (SEQ ID NO:6).--

Replace the paragraph beginning at page 13, line 9, with the following rewritten paragraph:

--**Fig. 15.** Schematic depiction of the intergenic region of the chimeric SK-FBD(4,5) gene (above: SEQ ID NO:8; below: SEQ ID NO:7) highlighting the design of a gly-gly-gly sequence,

Applicant : Rajesh Kumar, et al
Serial No. : 09/940,235
Filed : August 27, 2001
Page : 3

Attorney's Docket No.: 07064-009002

a transglutaminase cross-linking site and several unique restriction enzyme sites wherein different inter-genic cassettes can be conveniently swapped into this region. Also shown is the location of the natural Bsm I site in the SK gene which was exploited as the common junction point for joining the FBD sequences to the SK gene.--

Replace the paragraph beginning at page 13, line 23, with the following rewritten paragraph:

--**Fig. 17b.** DNA sequencing data of SK-FBD(4,5) hybrid cassette in T7 expression vector, pET23(d) (SEQ ID NO:9).--

Replace the paragraph beginning at page 13, line 33, with the following rewritten paragraph:

--**Fig. 19b.** DNA sequencing data of SK-FBD(1,2) hybrid cassette in T7 expression vector (SEQ ID NO:10).--

Replace the paragraph beginning at page 14, line 4, with the following rewritten paragraph:

--**Fig. 21b.** DNA sequencing data of FBD(4,5)-SK gene block as present in the T7 expression vector pET23(d)-FBD(4,5)-SK (SEQ ID NO:11).--

Replace the paragraph beginning at page 14, line 10, with the following rewritten paragraph:

--**Fig. 22b.** DNA sequencing data of FBD(4,5)-SK-FBD(4,5) gene block as present in the T7 expression vector pET23(d)FBD(4,5)-SK-FBD(4,5) (SEQ ID NO:12).--

Applicant : Rajesh Kumar, et al
Serial No. : 09/940,235
Filed : August 27, 2001
Page : 4

Attorney's Docket No.: 07064-009002

Replace the paragraph beginning at page 29, line 10, with the following rewritten paragraph:

--RG-7 (forward primer)

5'-ATT GCT GGA CCT GAG TGG CT-3' (SEQ ID NO:25)

(specific for the first seven codons of the SK gene; Cf Fig. 11)--

Replace the paragraph beginning at page 29, line 15, with the following rewritten paragraph:

--RG-6 (reverse primer)

5'-TGG TTT TGA TTT TGG ACT-3' (SEQ ID NO:26)

(specific for codons 57-62 of SK gene)--

Replace the paragraph beginning at page 32, line 4, with the following rewritten paragraph:

--SCI-I

5'-C ATG ATA GCT GGT CCT GAA TGG CTA CTA GAT CGT CCT TCT GTA AAT
AAC AGC C-3' (SEQ ID NO:13)

(Partial NcoI site)--

Replace the paragraph beginning at page 32, line 10, with the following rewritten paragraph:

--SC-II

5'-AA TTG GCT GTT ATT TAC AGA AGG ACG ATC TAG TAG CCA TTC AGG
ACC AGC TAT-3' (SEQ ID NO:14)

(Partial MfeI site)--

Applicant : Rajesh Kumar, et al
 Serial No. : 09/940,235
 Filed : August 27, 2001
 Page : 5

Attorney's Docket No.: 07064-009002

Replace the paragraph beginning at page 32, line 31, with the following rewritten paragraph:

--Mfe I primer: 5'-C-AGC-CAA-TTG-GTT-GTT-AGC-GTT-GCT-3' (SEQ ID NO:15)--

Replace the paragraph beginning at page 37, line 24, with the following rewritten paragraph:

--Forward primer (MY 13);

5'-CCG GAA TTC GCG CAA CAG ATT GTA CCC ATA GCT GAG AAG TGT TTT

Eco R1

Tansglutaminase-
recognition sequence

hybridizes to upstream
FBD(4,5) sequences

GA-3' (SEQ ID NO:16)--

Replace the paragraph beginning at page 37, line 30, with the following rewritten paragraph:

--Reverse primer (MY 14);

5'-GGC CTT AAG AGC GCT CTA ACG AAC ATC GGT GAA GGG GCG TCT A-3'

(SEQ ID NO:17)

'clamp'

Afl II

Eco 47 III

stop
codon

hybridizes to downstream
FBD(4,5) sequences--

Replace the paragraph beginning at page 38, line 27, with the following rewritten paragraph:

--The sequence of primer RG-3 is given below highlighting features incorporated in its design (bold letters denote non-hybridizing segments towards the 5'-end of the primer to distinguish these from the sequence complementary with respect to template DNA).

5'-G AAT GCT AGC TAC CAT TTA GCT GGT GGT GGC CAG GCG CAA CAG

Bsm I
(hybridizes to SK
gene at codons 376-383)

Bst X

Xcm I

Bal I

(-gly-gly-gly-)

segment hybridizing with the
5'-end of DNA block
from PCR-1 at the TG
recognition site

Applicant : Rajesh Kumar, et al
 Serial No. : 09/940,235
 Filed : August 27, 2001
 Page : 6

Attorney's Docket No.: 07064-009002

ATT GTA CCC-3' (SEQ ID NO:18)--

Replace the paragraph beginning at page 45, line 1, with the following rewritten paragraph:

--Upstream primer, MY-10

SK sequence (codons 377-383; Cf. Fig. 3)

5'-G-TAC-GGA-TCC G-AAT-GCT-AGC-TAT-CAT-TTA-GCG-GGT-GGT-GGT-

Bam HI

Bsm I

(gly-gly-gly-)

CAG-GCG-CAG-CAA-ATG-GTT-3' (SEQ ID NO:19)

hybridizes at the TG-recogntn. site just before the FBD sequences--

Replace the paragraph beginning at page 45, line 9, with the following rewritten paragraph:

--Downstream primer, MY-6

5'-GGC-CTT-AAG-AGC-GCT-CTA-TTA-GAT-GGT-ACA-GCT-TAT-TCT-3' (SEQ ID NO:20)

'clamp'

Eco R1
site

Eco 47 II

stop
codons

sequence hybridizing with FBD (1,2)
codons 99-104 (Cf.Fig.6)--

Replace the paragraph beginning at page 49, line 1, with the following rewritten paragraph:

--Upstream PCR-I primer KRG-8:

Transglutamate recognition site

5'-CC-ATG-GTG-CAA-GCA-CAA-CAG-ATT-GTA-CCC-ATA-GCT-GAG-AAG-
 Partial Nco I
 site

150 152 154

hybridizes to beginning
 of FBD(4) segment
 (codon numbers of FBD are
 shown as per Fig.6)

TGT-3' (SEQ ID NO:21)--

Applicant : Rajesh Kumai, et al
 Serial No. : 09/940,235
 Filed : August 27, 2001
 Page : 7

Attorney's Docket No.: 07064-009002

Replace the paragraph beginning at page 49, line 9, with the following rewritten paragraph:

--Downstream PCR-I primer KRG-9:

sequence complementary to codons 1-5 of SK (No.'s indicated below)

5'-CTC-AGG-TCC-AGC-AAT-ACG-AAC-ATC-GGT-GAA-GGG-GCC-AGA-T-3'
 5 4 3 2 1 259 257 255 253
 (SEQ ID NO:22)

sequence hybridizing with end of FBD(5) segment
 (No.'s indicated are codons, as per Fig.6).--

Replace the paragraph beginning at page 49, line 21, with the following rewritten paragraph:

--Upstream PCR-II primer, KRG-11

FBD(5) sequence, as overhang;
 Codon numbers (cf. Fig.6)
 are indicated

sequence hybridizing with
 SK gene; codon No.'s (Cf.
 Fig.3) are indicated

5'-TTC-ACC-GAT-GTT-CGT - ATT-GCT-GGA-CCT-GAG-TGG-CTG-CTA-GAC-3'
 255 257 259 1 3 5 7 9
 (SEQ ID NO:23)--

Replace the paragraph beginning at page 49, line 29, with the following rewritten paragraph:

--Upstream PCR-II primer, KRG-12

5'-TGG-TTT-TGA-TTT-TGG-ACT-TAA-GCC-TTG-3' (SEQ ID NO:24)
 62 60 58 56 54

Note: sequence hybridizing with SK gene (codon No.'s are indicated; see Fig. 3)--

In the drawings:

Substitute the enclosed 26 sheets of formal drawings filed herewith for the original informal drawings as filed with the application.

Applicant : Rajesh Kumar, et al
Serial No. : 09/940,235
Filed : August 27, 2001
Page : 8

Attorney's Docket No.: 07064-009002

REMARKS

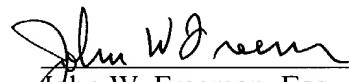
Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification, and replace the original informal drawings with formal drawings. No new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050, referencing attorney docket no. 07064-009002.

Respectfully submitted,

Date: 4/5/02



John W. Freeman, Esq.
Reg. No. 29,066

Fish & Richardson P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

“Version With Markings to Show Changes Made”

In the specification:

Paragraph beginning at page 12, line 7, has been amended as follows:

Fig. 3. DNA and protein sequence of streptokinase of *S. equisimilis* H46A (SEQ ID NOs:1 and 2, respectively) (GenBank accession number: gb/K02986/STRSKC).

Paragraph beginning at page 12, line 16, has been amended as follows:

Fig. 6. DNA and protein sequence of the gene-segment encoding for FBDs 1-5 of human fibronectin (SEQ ID NOs:3 and 4, respectively) (the DNA sequence has been obtained from EMBL; the file and accession no.'s are ID-HSFIBI and [X 02761,K 00799, K 02273,X 00307, X 00739] X02761, K00799, K02273, X00307, X00739).

Paragraph beginning at page 12, line 32, has been amended as follows:

Fig. 11. Nucleotide sequence of SK-NTRN gene (SEQ ID NO:5).

Paragraph beginning at page 12, line 34, has been amended as follows:

Fig. 12. Predicted secondary structure of native (A) and translationally silently modified (B) 5'-ends of the SK gene sequence (SEQ ID NOs:27 and 28, respectively).

Paragraph beginning at page 13, line 7, has been amended as follows:

Fig. 14. Nucleotide sequence of SK-NTR gene (SEQ ID NO:6).

Paragraph beginning at page 13, line 9, has been amended as follows:

Fig. 15. Schematic depiction of the intergenic region of the chimeric SK-FBD(4,5) gene (above: SEQ ID NO:8; below: SEQ ID NO:7) highlighting the design of a gly-gly-gly sequence, a transglutaminase cross-linking site and several unique restriction enzyme sites wherein different inter-genic cassettes can be conveniently swapped into this region. Also shown is the

Applicant : Rajesh Kumar, et al
Serial No. : 09/940,235
Filed : August 27, 2001
Page : 10

Attorney's Docket No.: 07064-009002

location of the natural Bsm I site in the SK gene which was exploited as the common junction point for joining the FBD sequences to the SK gene.

Paragraph beginning at page 13, line 23, has been amended as follows:

Fig. 17b. DNA sequencing data of SK-FBD(4,5) hybrid cassette in T7 expression vector, pET23(d) (SEQ ID NO:9).

Paragraph beginning at page 13, line 33, has been amended as follows:

Fig. 19b. DNA sequencing data of SK-FBD(1,2) hybrid cassette in T7 expression vector (SEQ ID NO:10).

Paragraph beginning at page 14, line 4, has been amended as follows:

Fig. 21b. DNA sequencing data of FBD(4,5)-SK gene block as present in the T7 expression vector pET23(d)-FBD(4,5)-SK (SEQ ID NO:11).

Paragraph beginning at page 14, line 10, has been amended as follows:

Fig. 22b. DNA sequencing data of FBD(4,5)-SK-FBD(4,5) gene block as present in the T7 expression vector pET23(d)FBD(4,5)-SK-FBD(4,5) (SEQ ID NO:12).

Paragraph beginning at page 29, line 10, has been amended as follows:

RG-7 (forward primer)

5'-ATT GCT GGA CCT GAG TGG CT-3' (SEQ ID NO:25)

(specific for the first seven codons of the SK gene; Cf Fig. 11)

Paragraph beginning at page 29, line 15, has been amended as follows:

RG-6 (reverse primer)

5'-TGG TTT TGA TTT TGG ACT-3' (SEQ ID NO:26)

(specific for codons 57-62 of SK gene)

Applicant : Rajesh Kumar, et al
 Serial No. : 09/940,235
 Filed : August 27, 2001
 Page : 13

Attorney's Docket No.: 07064-009002

Paragraph beginning at page 49, line 1, has been amended as follows:

Upstream PCR-I primer KRG-8:

Transglutamate recognition site

	150 152 154
5'-CC-ATG-GTG-CAA-GCA-CAA-CAG-ATT-GTA-CCC-ATA-GCT-GAG-AAG-	
Partial Nco I	hybridizes to [begining]
site	<u>beginning</u> of FBD(4) segment
	(codon numbers of FBD are
	shown as per Fig.6)

TGT-3' (SEQ ID NO:21)

Paragraph beginning at page 49, line 9, has been amended as follows:

Downstream PCR-I primer KRG-9:

sequence complementary to codons 1-5 of SK (No.'s indicated below)

	5 4 3 2 1 259 257 255 253
5'-CTC-AGG-TCC-AGC-AAT-ACG-AAC-ATC-GGT-GAA-GGG-GCC-AGA-T-3'	
(SEQ ID NO:22)	

sequence hybridizing with end of FBD(5) segment
 (No.'s indicated are codons, as per Fig.6).

Paragraph beginning at page 49, line 21, has been amended as follows:

Upstream PCR-II primer, KRG-11

FBD(5) sequence, as overhang;
 Codon numbers (cf. Fig.6)
 are indicated

sequence hybridizing with
 SK gene; codon No.'s (Cf.
 Fig.3) are indicated

	255 257 259 1 3 5 7 9
5'-TTC-ACC-GAT-GTT-CGT - ATT-GCT-GGA-CCT-GAG-TGG-CTG-CTA-GAC-3'	
(SEQ ID NO:23)	

Applicant : Rajesh Kumar, et al
Serial No. : 09/940,235
Filed : August 27, 2001
Page : 14

Attorney's Docket No.: 07064-009002

Paragraph beginning at page 49, line 29, has been amended as follows:

Upstream PCR-II primer, KRG-12

5'-TGG-TTT-TGA-TTT-TGG-ACT-TAA-GCC-TTG-3' (SEQ ID NO:24)

62 60 58 56 54

Note: sequence hybridizing with SK gene (codon No.'s are indicated; see Fig. 3)